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TITLE: Directing Spinal Cord Plasticity: The Impact of Stretch Therapy on Functional Recovery after Spinal Cord Injury.

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT Essentially all spinal cord injured patients receive stretching therapies beginning within the first few weeks post-injury. Despite this fact, almost nothing is known about how stretching might influence the neural circuitry in the spinal cord that is responsible for controlling the motor and locomotor activities of the legs. Recently, while studying activity-based rehabilitation in a rat model of spinal cord injury, we observed that stretching actually worsened locomotor recovery. The goal of this project is to investigate how the timing and intensity of a stretch-based therapy influences locomotor recovery after moderate and severe spinal cord injuries. In this, the first year of this award, we have found that stretching negatively influences locomotor function in animals with both acute (within days) and chronic (after 3 months) spinal cord injuries. We have also determined that stretching for short periods of time (4-5 weeks) allows substantial recovery to occur once stretching is stopped, and both acute and chronic animals show a similar time course of recovery. Finally, in very preliminary studies, we have found that the torque being applied during stretching of the rat hindlimb is roughly similar to that applied to human lower extremities relative to body weight.					
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Annual Report:

SC110169 – “Directing Spinal Cord Plasticity: The Impact of Stretch Therapy on Functional Recovery after Spinal Cord Injury”.

Principle Investigator: David S. K. Magnuson, PhD. University of Louisville.

Introduction:

This research focuses on the impact of stretching (physical therapy maneuvers involving force or torque applied to specific muscle groups) on functional recovery after spinal cord injury in a rat model. We have undertaken these studies because of an observation we made a few years ago during a study where the hindlimbs of rats with spinal cord injuries were being immobilized in a wheelchair. We found that immobilization dramatically influenced locomotor recovery, presumably, by reducing the sensory input associated with movement (Caudle et al., 2011). In that study we employed a stretching procedure designed to prevent reductions in joint range-of-motion. The stretching didn't prevent contractures, but it did have a negative impact on locomotor recovery. Thus, in the current study, specific Aim (SA) 1 focuses on the timing of stretching relative to the injury and whether or not there is a window of susceptibility to a stretching-based therapy. SA2 focuses on the pattern and forces of the actual stretching protocol and if the negative influence of stretching is due primarily to the length of each maneuver or to the forces applied during stretch. An overarching goal in the project is to gather the necessary pre-requisite information needed to determine how relevant this phenomenon is for humans with spinal cord injury and to prepare to translate these findings to the clinical situation.

Key Words: spinal cord injury, locomotor recovery, physical therapy, muscle stretch, joint range-of-motion, rat.

Overall Project Summary:

In this, the third year of this project, we have accomplished substantial components of Tasks 3-6 as described in the original Statement of Work. This has involved aspects of Specific Aims 1 & 2 as follows.

1. We completed the analysis of muscles from the acute-chronic study and found that there are no indications of frank muscle damage from stretching at either acute or chronic time points. We measured centralized nuclei, a marker of muscle damage/repair, and found no increase in muscles from acutely or chronically stretched compared to unstretched animals. See Table 1 and Figure 1.
2. We completed the analysis of muscle fiber size (cross sectional area or CSA) and found differences between injured and uninjured, and some differences between acutely and chronically stretched animals that were commensurate with their ability to step (Table 1).

Group	Muscle Fiber CSA (μm^2)		
	Tibialis Anterior	Gastrocnemius	Biceps Femoris
Acute Stretch (n=10)	573.05 \pm 99.00	730.04 \pm 139.75*	616.89 \pm 154.77*
Chronic Stretch (n=9)	531.52 \pm 116.70	598.83 \pm 122.42*^	468.38 \pm 100.84*^
Control (n=3)	521.55 \pm 82.26	871.96 \pm 261.74^	684.55 \pm 118.28^
Group	Centralized Nuclei Count (% of MF analyzed)		
	Tibialis Anterior	Gastrocnemius	Biceps Femoris
Acute Stretch (n=10)	3.53% \pm 0.020	3.457% \pm 0.022	1.669% \pm 0.008
Chronic Stretch (n=9)	2.90% \pm 0.016	2.738% \pm 0.012	3.294% \pm 0.035
Control (n=3)	2.01% \pm 0.014	3.456% \pm 0.018	4.283% \pm 0.033
Group	Collagen Area (mm^2)		
	Tibialis Anterior	Gastrocnemius	Biceps Femoris
Acute Stretch (n=10)	0.093 \pm 0.039	0.071 \pm 0.029	0.127 \pm 0.026
Chronic Stretch (n=9)	0.121 \pm 0.519	0.073 \pm 0.019	0.146 \pm 0.028
Control (n=3)	0.112 \pm 0.171	0.064 \pm 0.014	0.116 \pm 0.034

3. We continued the massive undertaking of analyzing the EMG patterns induced by stretching. We have finalized the system of analysis and categorization of the patterns and are almost finished the analysis. See below (Key Research Accomplishments)
4. We have established the technique of immunostaining for C-fos, an immediate-early onset gene that is expressed in neurons that are acutely active. The pattern of neuron activation after stretching will be extremely important, in particular if we find that it is distinct from other kinds of activity like stepping or swimming. This data is currently being analyzed and there are no results available to show.

M. Gastrocnemius

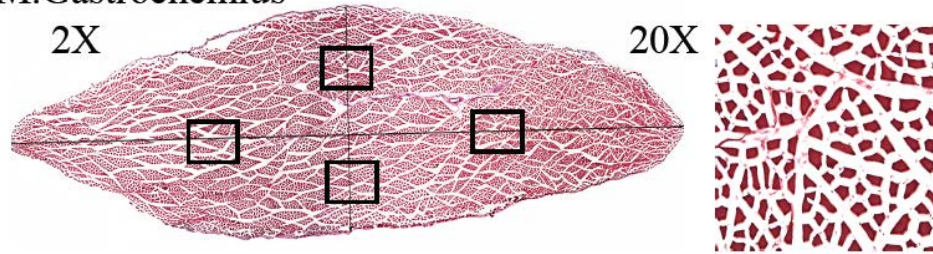


Figure 1. Shown is a medial gastrocnemius muscle in cross section and 4 mid-bell regions of interest chosen for counting based on the long and short axes. These are unbiased principles of counting.

Key Research Accomplishments.

The results described above are very significant steps towards completing the proposed studies and will provide critical information as we move forward. The primary research accomplishments overall for this third year of the project are:

1. Stereotypic and repeatable EMG patterns are induced in the limb contralateral to that being stretched. These patterns including clonus, spasms and air-stepping. All three patterns appear to differ in prevalence over time post-injury. These observations show that stretching is activating spinal circuitry that crosses the midline and that it induces patterns of activity commonly seen in spinal cord injured patients. These are critical issues to the translation of our findings to the clinic. See Figure 2.
2. That daily stretching, whether it be using a phasic (rhythmic) pattern, or the typically used (in the clinic) stretch and hold technique, is devastating to the capacity of the spinal cord to generate locomotor activity after injury. See Figure 3
3. That recovery from the negative impact of stretching is robust and similar for both a tonic and phasic pattern of stretching. We determined that phasic stretching is no more or less detrimental to locomotor recovery than is tonic stretching. See Figures 3 and 4.
4. That phasic (rhythmic) stretching does not induce frank muscle damage observable as an increase in the number of centralized nuclei in stretched muscles, nor does it induce an increase in the collagen (fibrosis), another indicator of muscle damage (data not shown).

These findings are extremely significant and will be strengthened as all the histological and immunohistochemical data is analyzed and we begin to relate the details of the forces, kinematics (joint angles) and EMG patterns for each of the muscle groups to changes in the spinal cord itself.

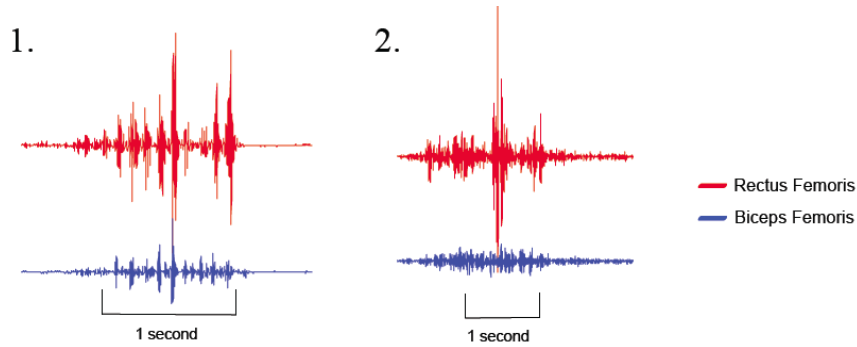


Figure 2. Shown are stereotypic patterns of clonus (1) and spasms (2) recorded from muscles in the limb contralateral to the one being stretched. The clonus pattern has a burst frequency of 6-12Hz and is often triggered when the stretch position is released. The spasms are typical co-contractions of antagonist muscles that are triggered at first movement, during the stretch or when the stretch position is released. These bursts are 1-5mV in amplitude. Thus study involved a group of 8 rats.

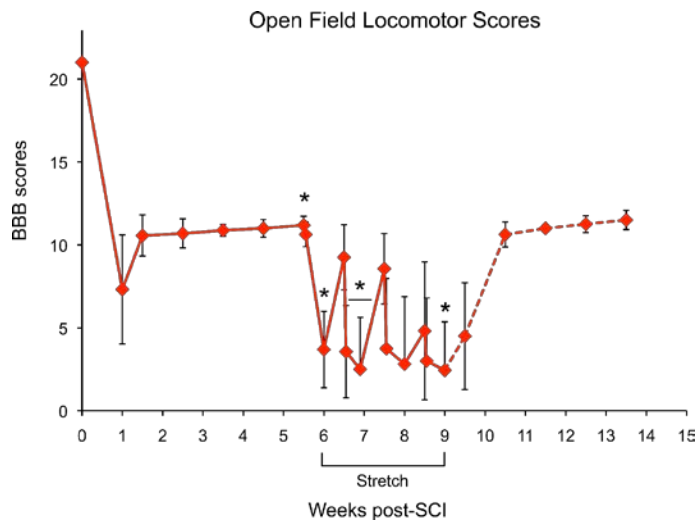


Figure 3. Shown are Open Field Locomotor Scores for hindlimb function during overground locomotion for animals with 25g-cm (moderately-severe) injuries at the T10 level. These animals received phasic stretching (2 secs on, 1 sec off) daily (M-F). The typical saw-tooth pattern of functional disruption is seen because we assess function on Monday mornings (before stretching), Monday afternoon 3-4hours after one stretching session and then on Friday afternoon 3-4hours after the final stretching session of the week (the lowest scores). As seen following stretching using our standard stretch-and-hold pattern, once stretching is stopped the locomotor capacity recovers rapidly over 1-2 weeks. This study involved a group of 8 rats.

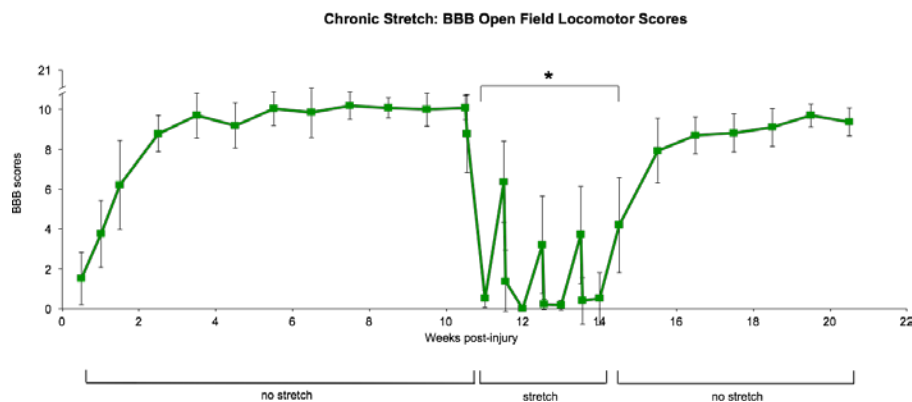


Figure 4. Shown are the Open Field Locomotor Scores for hindlimb function during overground locomotion for animals with 25g-cm (moderately-severe) injuries at the T10 level. These animals received our tonic stretch and hold pattern daily starting at 10 weeks post-injury. This graph is provided for comparison with Figure 3 (phasic stretching). These data are for a group of 9 rats.

Conclusion:

Our results so far are extremely important because they validate and extend what we found previously and begin to gather the information we need to move towards clinical translation. They demonstrate that the forces we are using while stretching the rats are not causing overt muscle damage and that the peak forces are occurring in response to nervous system activation, and not the stretching force itself. We are building up a picture of how the spinal cord is responding to the afferent input caused by the stretching and that at least some of this response involves co-activation at high-frequency and low amplitude. There continues to be solid rationale for proceeding with the remaining experiments as described in the original proposal. None of our results are pointing towards the development of a product, but will lead to the suggestion that our current stretching practices in the clinic will need to change. Our remaining experiments will help to illustrate how those practices should change.

Publications, abstracts and presentations:

This project has resulted in one full-length publication that was accepted last year but has now been published. The full reference is included below (Caudle et al., 2015). This paper reports on a study that was initiated prior to grant submission, but was completed after the grant was awarded. This project has also resulted in an abstract/poster presentation at the Society for Neuroscience meeting and the American Spinal Injury Association meetings in 2015. The title of the abstracts and author lists are shown below.

Inventions, Patents and Licenses: Nothing to report.

Reportable Outcomes: Nothing to report.

Other Achievements: Nothing to report.

References:

Caudle KL, Atkinson DA, Brown EH, Donaldson K, Seibt E, Chea T, Smith E, Chung K, Shum-Siu A, Cron C, Magnuson DSK. Hindlimb stretching alters locomotor function post-spinal cord injury in the adult rat. *Neurorehab and Neural Repair* 29(3): 268-77, 2015

Keller AV, Nord K, Wade A, Shum-Siu A, and Magnuson DSK. Electromyographic patterns in the contralateral limb in response to muscle stretch in rats with moderate spinal cord injuries. *Society for Neuroscience* 2015.

Keller AV, Nord K, Wade A, Shum-Siu A, and Magnuson DSK. EMG patterns of the contralateral limb in response to muscle stretch in rats with mild SCIs. *American Spinal Injury Association Meeting*, Montreal, PQ. 2015.

Appendix: Link to Caudle et al., 2015. Neurorehab and Neural Repair.

<http://nnr.sagepub.com/content/early/2014/08/07/1545968314543500.long>